Revision to Submission MRID No. 49605601 - Proposed Protocol to Support Use of EPA Reg. No. 90094-1 with the Mini Chlorine Dioxide System (MCS) to Sterilize/Decontaminate Confined Areas, Specifically Including Biological Safety Cabinets

July 15, 2015

Test Material

Sodium Chlorite Technical, EPA Reg. No. 90094-1 (Alternate trade name – "CD Generation Part "A")

Data Requirement

OCSPP Guideline 810.2100, Sterilants

Author

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Study Completion Date

To Be Determined

Testing Facilities

DRS Laboratories Inc. 450 Allentown Drive Allentown Pa. 18109

And

Azzur Labs LLC. 4125 Independence Drive, Suite 5 Schnecksville, PA 18078 Abby Roth

Laboratory Project / Guideline Number
To Be Determined

PROTOCOL SUMMARY

PURPOSE:

The purpose of this proposed study is to satisfy EPA Guideline 810.2100 as required to support the amendment of EPA Reg. No. 90094-1 to include use as a sterilant in confined spaces when applied using the DRS Laboratories' (DRS's) Mini Chlorine Dioxide System® (MCS) as directed.

BACKGROUND:

The label amendment is to add the use of this product to generate gaseous Chlorine Dioxide (CD) for use in the registrant's MCS to decontaminate enclosed area volumes up to 120 cubic feet to include biological safety cabinets.

Label Claims:

CD Generation Part "A" is for use to generate CD gas used to [decontaminate] [sterilize] [fumigate] non-porous and porous surfaces in sealed enclosures, confined spaces, rooms or areas, or vehicles located in government, industrial, manufacturing, fermentation, commercial and institutional microbiological laboratory settings, including human and animal research facilities and areas, cleanrooms; animal isolation rooms, necropsy suites, pass-throughs, airlocks, decontamination chambers, biological safety cabinets, glove boxes, isolators, incubators, animal cages and devices, laboratory equipment, supply and exhaust filter systems, and HEPA filtered devices.

DRS's CD gas generation system has been validated for use to [decontaminate] [sterilize] [fumigate] enclosures up to 120 cubic feet. Use to [decontaminate] [sterilize] [fumigate] larger enclosures can be done on a case-by-case basis if appropriate biological indicators (BI's) to confirm required performance are included in the treatment process. Uses other than those specified in the appropriate DRS equipment Instruction Manual are not permitted and may not be effective. Review and follow all DRS equipment Instruction Manual instructions and precautions on how to properly utilize this product and equipment.

METHOD

TESTING FACILITIES and GLP COMPLIANCE:

DRS Laboratories, Inc.:

The CD decontamination procedure will be performed at the facilities of DRS Laboratories, Inc. using a Baker SterilGard® III Advance Biological Safety Cabinet, Model SG:603 (see attached Operator's Manual), which has a total volume of 78 ft³ as the representative treatment area. DRS will not be making the claim to have conducted the decontamination process in full compliance with 40 CFR Part 160 as that facility is not a contract research laboratory. However, all efforts will be made to comply with GLP regulations. The first step in the BI recovery into the media tubes will also be performed at this site by Azzur.

Azzur Labs LLC:

Azzur Labs LLC will perform the analyses of the Biological Indicators (BI's) for growth using appropriate media and conditions for the respective BI's and in compliance with GLP regulations. The second step of incubation and reads of the BI's will be performed at the Azzur site by Azzur under GLP regulations.

MATERIALS:

TEST SUBSTANCE:

Sodium Chlorite Technical, EPA Reg. No. 90094-1 (Alternate trade name - CD Generation Part "A") Ingredients:

- 80% Sodium Chlorite
- 20% Inerts

Three lots of EPA Reg. No. 90094-1 will be tested at the lowest effective concentration of Chlorine Dioxide. The CD gas produced from the Sodium Chlorite is the sterilant used in conjunction with the DRS Laboratories MCS generator to generate and distribute CD gas.

The Sodium Chlorite, EPA Reg. No. 90094-1, is reacted with "Solid Component B", which contains Sodium Bisulfate and a proprietary secondary activator to generate CD gas.

THE DRS MCS APPLICATION SYSTEM INCLUDES:

- Control box with CD Generation, Recirculation, Scrub Blower(s)
- CD dispensing assembly, releasing valves and plumbing
- Charcoal Scrubber
- Supply, Return Recirculation Duct Lines
- Supply, Return Sealing Panel(s)
- Humidifier
- Temp / Humidity gauge

TREATMENT CONDITIONS:

Preparation of the area for decontamination is discussed in detail in DRS's MCS Generator's Owner's Manual.

TEMPERATURE:

The optimal temperature range for CD gas generation is 59-104°F (15-40°C). For this proposed study, the decontamination process will be conducted within the narrower and lower temperature range of 59°F to 75°F, which is representative of the least optimal ("worst case") conditions within the acceptable temperature range for CD gas generation.

RELATIVE HUMIDITY:

The optimal relative humidity range for CD gas generation is 60% to 85% RH. For this proposed study, the decontamination process will be conducted within the narrower and lower relative humidity range of 60% to 70% RH, which is representative of the least optimal ("worst case") conditions within the acceptable relative humidity range for CD gas generation.

DIFFERENTIAL PRESSURE:

The optimal pressure range for CD gas generation is consider to be \pm 0.005 W.C. or shall be neutral in pressure. To be monitored in all tests.

SOIL LOAD:

The Agency has indicated that the use of a soil load on the BI's is a waived requirement. The BI's will be obtained from a commercial supplier.

OTHER:

All lighting shall be turned off or dark.

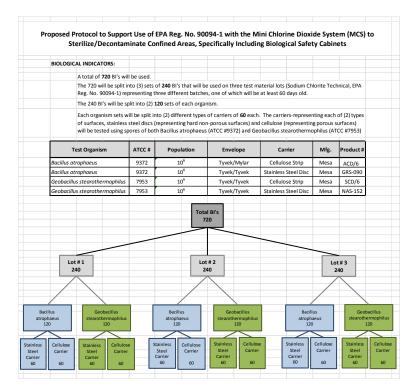
BIOLOGICAL INDICATORS:

As per the EPA Guideline OCSPP 810.2100, *Sterilants—Efficacy Data Recommendations* dated September 4, 2012, there is some discretion allowed as to what a sterilant should be tested against. The test organisms in the chart will be used because they have been utilized in other EPA field testing publically reported. Further, these BI's are commercially available and used routinely to monitor decontamination procedures in the field.

Test Organism	ATCC#	Population	Envelope	Carrier	Mfg.	Product #
Bacillus atrophaeus	9372	10 ⁶	Tyvek/Mylar	Cellulose Strip	Mesa	ACD/6
Bacillus atrophaeus	9372	10 ⁶	Tyvek/Tyvek	Stainless Steel Disc	Mesa	GRS-090
Geobacillus stearothermophilus	7953	10 ⁶	Tyvek/Tyvek	Cellulose Strip	Mesa	SCD/6
Geobacillus stearothermophilus	7953	10 ⁶	Tyvek/Tyvek	Stainless Steel Disc	Mesa	NAS-152

Based on the Agency's efficacy study review response (EPA Decision No. 488000) dated September 4, 2014, *Bacillus atrophaeus* (ATCC #9372) and *Geobacillus stearothermophilus* (ATCC #7953) appear to be acceptable test organisms to support a sterility claim for CD gas given testing on both stainless steel and paper carriers for both test organisms.

A total of 720 Bl's will be used. The 720 will be split into (3) sets of 240 Bl's that will be used on three test material lots (Sodium Chlorite Technical, EPA Reg. No. 90094-1) representing three different batches, one of which will be at least 60 days old. The 240 Bl's will be split into (2) 120 sets of each organism. Each organism sets will be split into (2) different types of carriers of 60 each. The carriers-representing each of (2) types of surfaces, stainless steel discs (representing hard non-porous surfaces) and cellulose (representing porous surfaces) will be tested using spores of both Bacillus atrophaeus (ATCC #9372) and Geobacillus stearothermophilus (ATCC #7953)



See attachment A: .EPA DRS Labs BI Test Quantity and Types.pdf

PLACEMENT OF BI's:

HEPA filtered devices such as a biological safety cabinet (BSC) are proposed as representing worse case conditions for an enclosed area being treated. Bl's will be placed inside the BSC at the following (28) locations:

Proposed Protocol to Support Use of EPA Reg. No. 90094-1 with the Mini Chlorine Dioxide System (MCS) to Sterilize/Decontaminate Confined Areas, Specifically Including Biological Safety Cabinets

BIOLOGICAL INDICATORS TEST LOCATIONS:

A total of 720 BI's will be used. The 720 will be split into (3) sets of 240 BI's that will be used on three test material lots (Sodium Chlorite Technical, EPA Reg. No. 90094-1) representing three different batches, one of which will be at least 60 days old. The 240 BI's will be split into (2) 120 sets of each organism. Each organism sets will be split into (2) different types of carriers of 60 each. The carriers-representing each of (2) types of surfaces, stainless steel discs (representing hard non-porous surfaces) and cellulose (representing porous surfaces) will be tested using spores of both Bacillus atrophaeus (ATCC #9372) and Geobacillus stearothermophilus (ATCC #7953)

Test Organism	ATCC #	Carrier	Qty. Total Per Test	Test Locations	Left Center Right	Qty. of Locations	Qty. Per Test location	ID#
Bacillus atrophaeus	9372	Cellulose Strip	60	Work Surface	Left	1	2	1-2
Bacillus atrophaeus	9372	Stainless Steel Disc	60	Work Surface	Ctr.	1	2	3-4
Geobacillus stearothermophilus	7953	Cellulose Strip	60	Work Surface	Right	1	2	5-6
Geobacillus stearothermophilus	7953	Stainless Steel Disc	60	Under Work Surface	Left	1	2	7-8
			Under Work Surface	Ctr.	1	2	9-10	
				Under Work Surface	Right	1	2	11-12
 			Rear Wall Return Plenum	Left	1	2	13-14	
		\	Rear Wall Return Plenum	Ctr.	1	2	15-16	
			Rear Wall Return Plenum	Right	1	2	17-18	
			Negative Pressure Plenum	Left	1	2	19-20	
				Negative Pressure Plenum	Ctr.	1	2	21-22
			1	Negative Pressure Plenum	Right	1	2	23-24
				Blower Impeller	Ctr.	1	6	25-30
				Positive Pressure Plenum	Left	1	2	31-32
			5	Positive Pressure Plenum	Ctr.	1	2	33-34
				Positive Pressure Plenum	Right	1	2	35-36
				Supply HEPA - Upstream	LHC	1	2	37-38
Po Po Po				Supply HEPA - Upstream	CTR	1	2	39-40
	/o _ [Supply HEPA - Upstream	RHC	1	2	41-42
				Supply HEPA - Downstream	LHC	1	2	43-44
•		0	#11	Supply HEPA - Downstream	CTR	1	2	45-46
E Grown type	4		4 -	Supply HEPA - Downstream	RHC	1	2	47-48
E CONCENTRATION TO THE PROPERTY OF THE PROPERT	- 1	9		Exhaust HEPA - Upstream Exhaust HEPA - Upstream	LHC CTR	1	2	49-50 51-52
Fig. 3 — Supply Pleaum		TO THE PARTY OF TH	-	Exhaust HEPA - Upstream	RHC	1	2	53-54
			_	Exhaust HEPA - Downstream	LHC	1	2	55-56
		-		Exhaust HEPA - Downstream	CTR	1	2	57-58
Notes: BSC blower is not operational during the test			Exhaust HEPA - Downstream	RHC	1	2	59-60	
		LHC: Left Hand	Corner		Totals	28	60	
		CTR: Center						
		RHC: Right Hand	l Corner					

DETERMINATION OF APPROPRIATE AMOUNT OF CD GAS IN DECON CYCLE:

As indicated above, CD gas is generated by combining *CD Generation Part "A"*, EPA Reg. No. 90094-1, with CD generation packet "B" in cold tap water. Absolute precision in the generation of CD is not realistic or obtainable and efforts to generate precisely the minimum amount required for a given situation can result in miscalculation and inadequate CD gas generation. Pre-measured packets of the "A" and "B" components are provided for use in 500 mL of cold tap water to generate and maintain CD gas to the concentration of 0.13 g/ft³ required for sterilization for the 90 minute treatment period. The validation of these CD gas generation values is presented in MRID No. 49320201.

The table below can be used to determine the number of packets of "A" and "B" appropriate for a given enclosure volume range to ensure adequate CD gas is generated. An equal number of packets of "A" and "B" are always used in combination with 500 mL of cold tap water.

Volume ft ³ (m ³)	BSC Size Width - ft ³ (m ³)	CD Generation Chemicals
0 (0.0) to 25 (0.7)	0-2 ft. (0.00-0.60)	1 each of A & B
25 (0.7) to 60 (1.7)	3-4 ft. (0.91-1.22)	2 each of A & B
60 (1.7) to 90 (2.5)	5-6 ft. (1.52-1.83)	3 each of A & B
90 (2.5) to 120 (3.4)	n/a – special	4 each of A & B

Note: There is variation in the amount of CD grams generated dependent upon the number of chemical packets used in relationship to the upper enclosure volume within the above ranges. It was found not to be a linear curve but following the above guideline table results in generation and maintenance of the minimum concentration required for sterilization, 0.13 g/ft³.

Since the test chamber for this study will be a Baker SterilGard® III Advance Biological Safety Cabinet, Model SG:603, which has a total volume of 78 ft³, 3 packets of *CD Generation Part "A"*, EPA Reg. No. 90094-1, and 3 packets of *CD generation Part "B"* will be used to generate the necessary CD gas for the decontamination/sterilization test.

Generation efforts shall be taken to generate the maximum CD gas concentration of 0.13 g/ft³, not to exceed this value. Generation will be performed with the three sets of Part "A" and "B" and when the value of 0.13 g/ft³ of CD is met, the CD generation shall cease and recirculation shall continue. (Note: hardness of the tap water to be also measured and documented).

DECONTAMINATION PROCESS: (GENERATION CYCLE):

A humidity source, temp / humidity meter, and biological indicators are placed within the enclosure to be decontaminated. The enclosure is then sealed incorporating into the seal a gas inlet and outlet port for use with the MCS. After appropriate humidity (i.e., within 60% to 70% RH) and temperature (i.e., within 59°F to 75°F) are confirmed, CD is produced and released and the decontamination cycle begins.

The test material will be used according to DRS's MCS instructions (see owner's manual). The method of CD gas generation utilizes *CD Generation Part A* in cold tap water with addition of *CD Generation Part B*, a solid acid, to generate CD gas. CD gas at a maximum concentration of 0.13g/ft³ CD gas will be generated and maintained within the treatment enclosure volume. The duration of the decontamination period will be a fixed time of 90 minutes.

The CD gas concentration will be continuous monitored using a CD Photometer Model CDP101, which will "act as" color or chemical indicators, which has the following characteristics:

- Measurement Range of 0.00 to 50 mg/l (0.00 to 18,100 ppm),
- Resolution: < ± 0.05 % of respective measuring range, and
- Repeatability: < ± 0.5 % of respective measuring range.

Measurements will be recorded during the decontamination process at 0, 10, 20, 30, 60, and 90 minutes as well as at 10, 20, and 45 minutes during the final scrubbing cycle.

There shall be no air recirculation by the BSC's internal blower. This will simulate worst case scenario as if there was a BSC blower failure. All recirculation of the CD gas will be performed by the DRS MCS CD generation equipment.

DECONTAMINATION PROCESS: (SCRUBBING CYCLE):

After a treatment period of 90 minutes, CD gas is removed from the enclosure using the MCS's "scrubbing cycle", which circulates the air in the enclosed area through an activated charcoal bed that captures the CD gas. The air inside the treatment area is sampled using a C16 PortaSens II CD gas detector, which has an accuracy of ± 5% and sensitivity of 1% of sensor module. The CD gas level must be below the OSHA PEL limit of 0.1 ppm for the scrubbing cycle can be considered complete. There should also be no noxious odors present; otherwise, the scrubbing cycle will need to be extended until that issue is eliminated. The scrubbing cycle generally takes about 45 minutes and should represent the removal of the CD gas on the BI's.

POST DECON / SCRUB - BI RECOVERY:

After the scrubbing cycle, the enclosure is disengaged from the MCS unit and the BSC will be unsealed, disassembled to recover all the BI's from the (28) locations. (It will take up to 10 - 15 to totally disassemble the BSC with recovery of immediately available BI's, like the ones inside the work surface or under the work surface areas to be processed first to save or reduce recovery and aseptic process times). DRS to perform.

At these times, all the BI's will immediately be aseptically processed into the neutralizing subculture media tubes (Tryptic Soy Broth (TSB) with 0.6% sodium thiosulfate) on site (DRS Laboratories) of the decontamination process by Azzur Labs. It is anticipated that Azzur Labs will initiate the process from removing the BI carriers from the BI envelope within minutes of their recovery from the treated BSC. Transfer all test samples before transferring the positive controls.

After all the BI's are processed into the neutralizing subculture media tubes, they will be transported to the Azzur laboratory, by Azzur to be placed in the appropriate temperature incubators.

INCUBATION AND TESTING OF THE BI'S:

BI's are aseptically transferred to the tubes of Tryptic Soy Broth (TSB) with 0.6% sodium thiosulfate or another appropriate medium, as determined by the neutralization verification. Positive controls will be unexposed BI's, one of each of the carrier types. Transfer all test samples before transferring the positive controls. *Geobacillus stearothermophilus* carriers are incubated at 55°C to 60°C. *Bacillus atrophaeus* carriers are incubated at 30°C to 35°C. Incubate appropriately and include tubes of the chosen growth medium without any indicators in each incubator to act as the negative controls. Visually inspect the tubes for turbidity at 24 hours to 4 days. Return the tubes to the appropriate incubator. Visually inspect the tubes for turbidity after at least seven days of incubation.

PERFORMANCE CRITERIA:

Evaluation of sterilant success shall be the product shall kill the test spores on all of the 720 BI's (carriers) without any failures (e.g., growth of test organism after treatment). Test samples and negative controls are clear (negative); positive controls are turbid (positive).

NEUTRALIZATION CONFIRMATION CONTROLS: LAB MATERIALS:

- 10 mL tubes of Tryptic Soy Agar with 0.6% Sodium Thiosulfate
- <100 CFU culture of Bacillus atrophaeus (ATCC 9372)
- <100 CFU culture of Geobacillus stearothermophilus (ATCC 7953)
- Cellulose Carriers without organisms
- Stainless Steel carriers without organisms

NEUTRALIZATION CONFIRMATION CONTROLS:

To ensure that the chlorine dioxide is effectively neutralized, a neutralization verification will be performed. The growth media shall contain a neutralization agent such as sodium thiosulfate; this is to eliminate the additional processing step of transferring the BI's into a sterile solution of sodium thiosulfate prior to placing the BI's into the media. Four blank cellulose carriers and four blank stainless steel carriers are exposed to the chlorine dioxide cycle. Immediately after the cycle, the carriers are aseptically removed from their envelopes and each is transferred to a 10 mL tube of TSB 0.6% sodium thiosulfate. Two tubes of each carrier type are inoculated with <100 CFU of Geobacillus stearothermophilus and two of each carrier type are inoculated with <100 CFU of Bacillus atrophaeus. Negative and positive controls are also run. Two of each type of carrier are aseptically transferred to a 10 mL tube of TSB 0.6% sodium thiosulfate, to serve as negative controls. Two of each type of carrier are aseptically transferred to 10 mL tubes of TSB 0.6% sodium thiosulfate. One tube with stainless steel and one tube with cellulose are inoculated with <100 CFU of Geobacillus stearothermophilus and one tube with stainless steel and one tube with cellulose are inoculated with <100 CFU of Bacillus atrophaeus. These will act as the positive controls. Geobacillus stearothermophilus carriers are incubated at 55°C to 60°C. Bacillus atrophaeus carriers are incubated at 30°C to 35°C. At less than or equal to seven days of incubation, the exposed tubes and the positive controls must be turbid. The negative controls must be clear. If the exposed BI tubes are not turbid, the neutralization of the chlorine dioxide was not effective and this testing is repeated with a different neutralizer. If any of the controls do not perform as expected, it will be investigated and the testing will be repeated.

PREPARATION OF CONTROLS:

Population Verification of the Biological Indicators

The population of each lot of biological indicators used in the study is verified prior to use. The verification will be performed according to the manufacturer's instructions.

Acceptance Criteria: Biological Indicators must yield a population of at least 1.0 x 10⁶.

Quality Control Testing of Media

The sterility and growth promotion capability of the TSB 0.6% sodium thiosulfate used in testing is verified prior to use. Sterility of the TSB 0.6% sodium thiosulfate is verified at the incubation temperatures used in testing.

<u>Acceptance Criteria</u>: Media must meet the criteria indicated on the manufacturer's certificate of analysis for growth promotion and sterility.

Test Controls

Two unexposed BI's of each type used in testing are submitted as controls with the test samples from each trial. A negative control is included by incubating an unopened tube of the same lot of TSB that was used for the test. The positive and negative controls are tested in the same manner as the test samples.

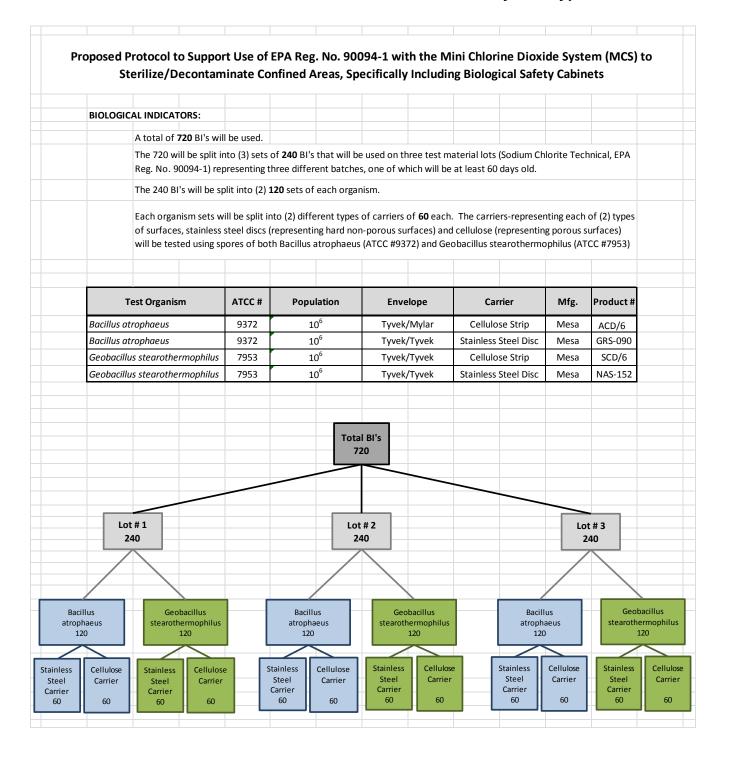
<u>Acceptance Criteria</u>: For the positive control, both unexposed controls for each lot of biological indicator used must be turbid (positive). For the negative control, the unopened tube of TSB must be clear (negative).

REFERENCES:

The references below may be consulted for additional background information.

- (1) Official Methods of Analysis of the AOAC International, Chapter 6, Disinfectants, Official Method 966.04 Sporicidal Activity of Disinfectants, Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.
- (2) Official Methods of Analysis of the AOAC International, Chapter 6, Disinfectants, Official Method 2008.05 Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of Bacillus subtilis on a Hard Nonporous Surface), Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.
- (3) Annual Book of ASTM Standards, Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides, Designation E 2197. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

ATTACHMENT A: .EPA DRS Labs BI Test Quantity and Types



ATTACHMENT B: .EPA BI Test Locations and Quantity

Proposed Protocol to Support Use of EPA Reg. No. 90094-1 with the Mini Chlorine Dioxide System (MCS) to Sterilize/Decontaminate Confined Areas, Specifically Including Biological Safety Cabinets

BIOLOGICAL INDICATORS TEST LOCATIONS:

A total of **720** BI's will be used. The 720 will be split into (3) sets of 240 BI's that will be used on three test material lots (Sodium Chlorite Technical, EPA Reg. No. 90094-1) representing three different batches, one of which will be at least 60 days old. The 240 BI's will be split into (2) 120 sets of each organism. Each organism sets will be split into (2) different types of carriers of 60 each. The carriers-representing each of (2) types of surfaces, stainless steel discs (representing hard non-porous surfaces) and cellulose (representing porous surfaces) will be tested using spores of both Bacillus atrophaeus (ATCC #9372) and Geobacillus stearothermophilus (ATCC #7953)

ATCC #	Carrier	Qty. Total Per Test	Test Locations	Left Center Right	Qty. of Locations	Qty. Per Test location	ID#
9372	Cellulose Strip	60	Work Surface	Left	1	2	1-2
9372	Stainless Steel Disc	60	Work Surface	Ctr.	1	2	3-4
7953	Cellulose Strip	60	Work Surface	Right	1	2	5-6
7953	Stainless Steel Disc	60	Under Work Surface	Left	1	2	7-8
La c			Under Work Surface	Ctr.	1	2	9-10
			Under Work Surface	Right	1	2	11-12
¬		_	Rear Wall Return Plenum	Left	1	2	13-14
	H H	_	Rear Wall Return Plenum	Ctr.	1	2	15-16
J		\	Rear Wall Return Plenum		1	2	17-18
		7	Negative Pressure Plenum		1	2	19-20
			Negative Pressure Plenum		1	2	21-22
	1 1 1 4 4	1	Negative Pressure Plenum		1	2	23-24
		-	Blower Impeller		1	6	25-30
1			Positive Pressure Plenum	Left	1	2	31-32
		5	Positive Pressure Plenum		1	2	33-34
			Positive Pressure Plenum		1	2	35-36
			Supply HEPA - Upstream		1	2	37-38
	6	- 1	Supply HEPA - Upstream		1	2	39-40
~			Supply HEPA - Upstream	RHC	1	2	41-42
·			Supply HEPA - Downstream	LHC	1	2	43-44
·	0	11 1	Supply HEPA - Downstream	CTR	1	2	45-46
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eration	al during the test		Exhaust HEPA - Downstream	RHC	1	2	59-60
		Corner		Totals	28	60	
	CTR: Center						
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